

primer "antisense" (CGGGATCCTTAAGGAGAGCTGTCATTTTCT (SEQ Id. No. 2)) and 5U Pfu DNA Polymerase from Strategene (La Jolla, CA, USA). The reaction was carried out for 26 cycles in the cycler following this scheme: 1' at 95°C, 1' at 60°C and 2' at 72°C. At the end of the reaction 100 µl of a 25:24:1 phenol chloroform and isoamyl alcohol solution were added and after extraction, DNA was precipitated overnight at 20°C in the presence of ethanol. After centrifugation, DNA was resuspended in 100 µl water and then subcloned in the pMOS vector (Amersham Italia, srl, Italy) according to the manufacturer's instructions contained in the kit "pMOS blunt end cloning kit". The resulting recombinant plasmid was amplified and sequenced, then digested with BamHI whose recognition site (G/GATCC) was present in both PCR primers' ends. Therefore the fragment was subcloned in the BamHI site of the retroviral vector PINCO VUOTO. The retroviral vector PINCO VUOTO had been previously obtained following excision with EcoRI and NotI of a 1441 bp fragment containing the CMV promoter (Cytomegalovirus) and the EGFP (enhanced green fluorescent protein) gene from the plasmid PINCO (F.Grignani et al., Cancer Res., 58, 14-19, 1998). After excision of the EcoRI-NotI fragment, the plasmid was closed after end blunting with Klenow fragment and called VINCO VUOTO. Such retroviral vector is now of 11448 bp in length.--